

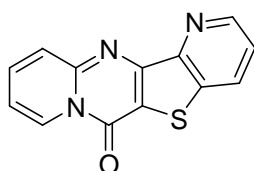
New potential antitumoral fluorescent tetracyclic thieno[3,2-*b*]pyridine derivatives: Interaction with DNA and encapsulation in nanoliposomes

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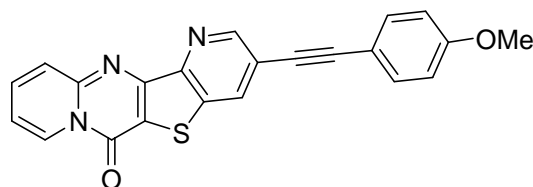
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Nanosized liposomes are among technological delivery methods for chemotherapeutic drugs in the treatment of cancer. This technique can potentially overcome many common pharmacologic problems, such as those involving solubility, pharmacokinetics, in vivo stability and toxicity [1,2]. Liposomes are closed spherical vesicles consisting of a lipid bilayer that encapsulates an aqueous phase in which hydrophilic drugs can be stored, while water insoluble compounds can be incorporated in the hydrophobic region of the lipid bilayer [3].

In this work, two new potential antitumoral fluorescent tetracyclic thieno[3,2-*b*]pyridine derivatives **1** and **2** (structures shown below), previously synthesized by some of us [4], were encapsulated in nanosized liposomes of DPPC (dipalmitoyl phosphatidylcholine), egg lecithin (phosphatidylcholine from egg yolk) and DODAB (dioctadecyldimethylammonium bromide). The phospholipids DPPC and egg lecithin (Egg-PC) are neutral components of biological membranes, while cationic liposomes based on the synthetic lipid DODAB have been used as vehicles for DNA transfection and drug delivery [5].



Compound 1



Compound 2

The investigation based on DNA interactions has a key importance in order to understand the mechanisms of action of antitumor and antiviral drugs and to design new DNA-targeted drugs [6]. Both compounds **1** and **2** were tested for their interaction with salmon sperm DNA using spectroscopic methods. These studies allowed to determine the binding constants and binding site sizes (in base pairs), through the McGhee and von Hippel modification of Scatchard plot [7] (Equation 1 and Table 1),

$$\frac{r}{c_f} = K_i (1 - nr) \left[\frac{(1 - nr)}{(1 - (n - 1)r)} \right]^{n-1} \quad (1)$$

where K_i is the intrinsic binding constant, n is the binding site size, r is the ratio $c_b/[DNA]$, c_b and c_f are the concentrations of bound and free compound, respectively. Fluorescence quenching experiments using external quenchers are also very useful to establish the DNA-binding modes, since intercalated chromophores are less accessible to anionic quenchers due to electrostatic repulsion with negatively charged DNA [8]. Quenching measurements using iodide ion showed that both compounds exhibit some intercalation in DNA, compound **2** being the more intercalative one, with a lower fraction of molecules accessible to quencher (Table 1).

Table 1. Binding constants to DNA (K_i), binding site sizes (n) and fraction of compound molecules accessible to external quenchers (f_a)

	K_i (M^{-1})	n (base pairs)	f_a
Compound 1	$(8.7 \pm 0.9) \times 10^3$	11 ± 3	0.89
Compound 2	$(5.9 \pm 0.6) \times 10^3$	7 ± 2	0.65

Considering the antitumoral potential of these compounds, the encapsulation in nanoliposomes is important for future drug delivery assays. Monodisperse and nanosized liposomes were prepared by injection of an ethanolic solution of the lipid in an aqueous media under vigorous stirring, above the lipid melting transition temperature ($T_m \sim 41\text{ }^\circ\text{C}$ for DPPC and $45\text{ }^\circ\text{C}$ for DODAB). The hydrodynamic diameters of $87 \pm 11\text{ nm}$ for DPPC, $51 \pm 2\text{ nm}$ for Egg-PC and $268 \pm 37\text{ nm}$ for DODAB were obtained by dynamic light scattering.

Fluorescence experiments of both compounds encapsulated in nanosized liposomes were carried out (Figure 1), in both gel (below T_m) and liquid-crystalline (above T_m) phases. Fluorescence anisotropy measurements allowed to conclude that compound **1** prefers an hydrated environment in the nanoliposomes, while compound **2** can be transported in the hydrophobic region of the lipid bilayer, especially in Egg-PC nanoliposomes.

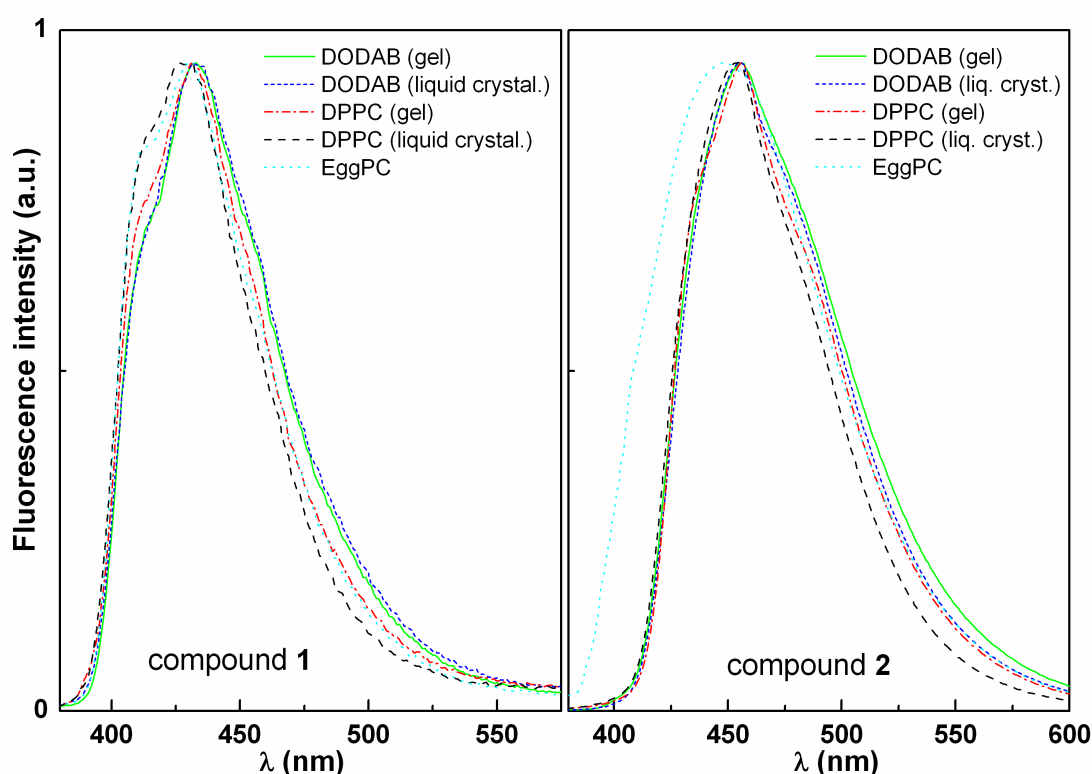


Figure 1. Normalized fluorescence emission spectra of compounds **1** and **2** incorporated in nanoliposomes of DPPC, Egg-PC and DODAB.

Acknowledgements

This work was funded by FCT-Portugal and FEDER through CFUM, CQ-UM, Project PTDC/QUI/81238/2006 cofinanced by FCT and program FEDER/COMPETE (FCOMP-01-0124-FEDER-007467) and PhD grants of M.S.D. Carvalho (SFRH/BD/47052/2008) and R.C. Calhela (SFRH/BD/29274/2006).

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